CULTURE CONDITIONS AFFECTING INDUCTION AND REGENERATION IN ISOLATED MICROSPORE CULTURES OF DIFFERENT *BRASSICA* SPECIES

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ABSTRACT

High yield and good quality of embryoids were obtained from cultures of isolated microsores of 11 different *Brassica napus, B. campestris* and *B. oleracea* genotypes. Different incubation temperatures, various induction and regeneration media were tested for their suitability to induce embryogenesis and plantlet regeneration. In most cases, an incubation pretreatment for 18 h at 35 °C followed by 10 days at 30 °C increased the number of embryoids per bud considerably. Addition of 0.1 ml of a suspension containing 1 % activated charcoal and 0.5 % agarose to each petri dish did not increase the number of embryoids per bud, the development of embryoids, however, was enhanced and the plantlet regeneration was favoured. The yield and quality of embryoids and plantlets were dependent on the genotype of the plant, the induction temperature, and the composition of incubation and regeneration media. Varying these factors sufficient numbers of embryoids and green plantlets could be obtained from any of the genotypes tested.

INTRODUCTION

The most effective way to produce haploids has been through androgenesis, by means of isolated microspore cultures. Although such haploids have been obtained from several crop species, the practical utilization of haploids in breeding programmes is limited to those species that are able to generate haploid plantlets in large numbers. The two major problems that limit the effective utilization of microspore culture are low embryoid yield and poor plant regeneration from microspore-derived embryoids.

Since the first report of isolated microspore culture in *B. napus* (Lichter, 1982), there has been remarcable progress in developing this system. Numerous factors influencing embryogenesis have been evaluated and conditions optimized. These include culture medium, growing condition of donor plants, genotype and microspore developmental stage (Gland *et al.*, 1988; Lichter, 1989; Hansen and Svinnset, 1993).

The efficient embryogenesis and plantlet regeneration from microspore cultures of *B. napus, B. campestris* and *B. oleracea* is reported in this paper. Special attention was given to the high temperature treatment on initiation of microspore culture, aeration of the culture vessels, different induction and regeneration media.

EXPERIMENTAL

Donor plants

Donor plants were grown in a climatized greenhouse at 12 - 15 °C in the presence of light

and 8 - 10 °C in the dark. Supplementary illumination was provided by mercury vapour lamps to insure an overall day length of 16 h. Complete fertilizer was applied every 3 - 4 days to maintain optimum growth. A total of 11 *Brassica* entries were used in the experiments:

B. napus:	Ag 902 and Ag 903, inbred lines from cv. 'Egra' and cv. 'Bronowski', Ag 9525, F_1 (resynthesized *B. napus* x microspore derived line), Ag 9526, F_1 (reciprocal cross).
B. campestris:	No. 43 (ssp. *pekinensis*), No. 170 (ssp. *narinosa*) Ag 46 and Ag 47 F_1 of *B. campestris* cross combinations.
B. oleracea:	No. 2169 and No. 2317 (var. *capitata*) cv. 'Braunschweiger' and cv. 'Stone Head', No. 2288 (var. *acephala*) cv. 'Cavalier Rouge'.

Induction and regeneration

NLN 82 medium, modified B 5 medium, A 14, A 47 and A 75, containing 12 % sucrose, titrated to pH 5.8 and filter sterilized, were used as induction media. 0.1 ml of a suspension of activated charcoal in agarose was added to 1.5 ml liquid medium of each culture dish. The petri dishes were incubated for 10 days at 30 °C or 35 °C only for 18 h and then transferred to 30 °C in dim light for the remainder of the incubation period (Gland *et al.*, 1988).

Under conditions of aeration the temperature was kept at 25 °C. Aeration was performed by agitating the culture dishes on a specially designed shaker with a rocking motion that allowed alternative sides of the proembryoid culture to have contact with the air, four times every minute. For most of the media a treatment of 35 °C for 18 h resulted in a greater number of embryoids (Table 1). Some of the individual results were inconsistent and for the NLN 82 medium incubation at 30 °C gave more embryoids than at 35 °C.

Efficient yields of embryoids were obtained when aeration was started about ten days after the incubation. When the embryoids became green and had developed to the torpedo shape or even the walking stick stage 5 or 6 were transferred to a new liquid medium consisting of half strength B 5 medium containing only 2 % sucrose and excluding growth regulators (Gland *et al.*, 1988). After 10 to 15 days the cotyledonary embryoids were transferred onto modified solid MS or B 5 media for regeneration.

R 1: Macro- and micronutrients of MS medium
R 2: Half strength of macro- and micronutrients of MS medium
R 3: Macro- and micronutrients of B 5 medium
R 4: Half strength of macro- and micronutients of B 5 medium

The four media were supplemented with 0.5 mg l^{-1} NAA, 1 g l^{-1} activated charcoal, 20 g l^{-1} sucrose and solidified with 3 g l^{-1} gelrite. The final pH of the media was generally 5.8.

Regeneration media R 2 and R 4 with only half strength of macro- and micronutrients of M S or B 5, respectively, gave much better results than both media with full strength of macro- and micronutrients.

The efficiency of regeneration of *B. campestris* was less than those of *B. napus* and *B. oleracea*.

TABLE 1: Influence of genotype, culture medium and incubation temperature on microspore embryogenesis in four *B. napus*, four *B. campestris* and three *B. oleracea*.

Genotype	No. of buds treated	Number of embryoids per bud									
		NLN 82		B 5		A 14		A 47		A 75	
		30°	35°	30°	35°	30°	35°	30°	35°	30°	35°
B. napus L.											
Ag 903	154	62	13	25	14	17	25	9	149	98	465
Ag 902	56	71	24	40	68	33	71	26	80	66	217
Ag 9525	126	137	28	72	35	52	48	18	47	61	195
Ag 9526	148	86	19	47	36	41	58	21	87	42	105
B. campestris L.											
43	42	3	1	4	5	6	11	2	4	3	11
170	94	8	3	2	6	2	3	3	4	2	9
Ag 46	78	32	17	11	17	18	24	13	16	31	89
Ag 47	82	24	13	11	16	14	21	7	48	10	67
B. oleracea L.											
2169	40	3	2	1	3	2	7	2	3	9	23
2288	30	5	2	2	2	2	13	3	11	7	26
2317	48	16	7	7	12	10	15	8	17	14	33

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